

CLAIMS

1. A method of obtaining a recombinant glucose binding protein expressed in non-plant host cells comprising reducing the glycogen content of a lysate of said cells.
2. A method as claimed in claim 1 comprising treating a lysate of said cells with
5 a buffer in which glycogen is soluble, but in which said protein is insoluble.
3. A method as claimed in claim 2 wherein other impurities are also soluble in said buffer.
4. A method as claimed in claim 2 or claim 3 wherein said buffer is a low ionic strength buffer ($I < 0.3$) with a pH between 8.5 and 9.5.
- 10 5. A method as claimed in claim 4 wherein said buffer further comprises a metal chelating agent.
6. A method as claimed in claim 5 wherein said metal chelating agent is EDTA.
7. A method as claimed in any one of claims 1 to 5 wherein said buffer further comprises a non-ionic detergent.
- 15 8. A method as claimed in claim 7 wherein said non-ionic detergent is Triton X-100.
9. A method as claimed in any one of claims 1 to 8 wherein said buffer comprises 2-(cyclohexylamino)-ethanesulphonic acid.
10. A method as claimed in any one of claims 1 to 8 wherein said buffer
20 comprises borate.
11. A method as claimed in claim 10 wherein said buffer is 20 mM Borax ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$).
12. A method as claimed in any one of claims 2 to 11 wherein said pH is between 9.05-9.25.

13. A method as claimed in any one of claims 2 to 12 wherein $I < 0.1$.
14. A method as claimed in any one of claims 1 to 13 further comprising the step of removing any glycogen-Con A complex formed.
15. A method as claimed in any one of claims 1 to 14 wherein said non-plant host
5 is a bacterium.
16. A method as claimed in claim 15 wherein said bacterium is *Escherichia coli*.
17. A method as claimed in claim 15 wherein said *Escherichia coli* cells are incapable of producing glycogen due to defects or mutations in genes for the biosynthesis of glycogen.
- 10 18. A method as claimed in any one of claims 1 to 17 wherein said non-plant host cells have been cultured in the absence of an assimilable carbohydrate or carbon source that may be accumulated as glycogen.
19. A method as claimed in claim 18 wherein said non-plant host cells have been cultured in the absence of glucose.
- 15 20. A method as claimed in any one of claims 1 to 19 wherein said glucose binding protein is a glucose binding lectin.
21. A method as claimed in claim 20 wherein said lectin is Concanavalin A.
22. A protein isolated by a method as defined in any one of claims 1 – 21.
23. The use of a buffer in which glycogen is soluble, but in which a glucose
20 binding protein is insoluble in the purification of a recombinant glucose binding protein expressed by a non-plant host cell.
24. The use as claimed in claim 23 modified by any of the features as claimed in any one of claims 2 – 20.

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25. A recombinant glucose binding protein that is substantially free of glycogen, and optionally other impurities.
26. A protein as claimed in claim 25, wherein said protein is a lectin.
27. A protein as claimed in claim 26, wherein said lectin is Concanavalin A, or a precursor form, or a mutant, or a variable valency or low valency form thereof.
28. The use of a recombinant glucose binding protein obtained by a method of claims 1-21 or a recombinant glucose binding protein as claimed in claim 25 in a system where the presence of glycogen would interfere with the binding of said glucose binding protein to another ligand.
29. The use as claimed in claim 28 for measuring glucose concentration.
30. The use as claimed in claim 28 or claim 29 wherein the recombinant protein is expressed from a coding sequence derived from a leguminous plant.
31. The use as claimed in claim 30 wherein said plant is of the genus *Canavalia*.
32. The use as claimed in any one of claims 28 to 31 wherein said plant is *Canavalia ensiformis*.
33. The use as claimed in any one of claims 28 to 32 wherein said protein is a lectin.
34. The use as claimed in any one of claims 28 to 32 wherein said protein is a Concanavalin-A like lectin.
35. The use as claimed in any one of claims 28 to 32 wherein said protein is Concanavalin A, or a precursor form, or a mutant, or a variable valency or low valency form thereof.
36. The use as claimed in claim 35 wherein said Concanavalin A is substantially free of Con-A-sequence related polypeptides or fragments.

37. The use as claimed in claim 35 or claim 36 wherein said Concanavalin A is in the mature tetrameric tetravalent form.
38. The use as claimed in any one of claims 29 to 37 wherein the protein is substantially free of glycogen.
- 5 39. The use as claimed in any one of claims 29 to 38 wherein said glucose concentration is measured by viscometric methods.
40. The use as claimed in any one of claims 29 to 38 wherein said glucose concentration is measured using a fluorescence-based method.
41. The use as claimed in any one of claims 29 to 40 wherein the method utilises
- 10 an analyte analogue which is a glucose derivative, a polymer or polysaccharide containing glucose or a carrier molecule covalently linked to a glucose derivative or glucose.
42. The use as claimed in claim 41 wherein said carrier molecule is a protein.
43. The use as claimed in claim 42 wherein said carrier protein is a serum
- 15 albumin.
44. The use as claimed in any one of claims 28 to 43 wherein said protein forms part of a glucose biosensor.